fauna [3]). Some might suggest that the island home of every member of the relevant trans-Atlantic clade (Hispaniola, Fuerteventura, Mallorca, Menorca, Elba) would actually imply that this lineage was an active and successful disperser at times, instead of being only a passive passenger on tectonic plates.

Rather than providing a definitive answer, our results and conclusions highlight the difficult nature of some of biology's big questions. Given the rapid substitution rates (in both our and Bauzà-Ribot *et al*. [2] analyses) and the great age of the question being considered, slower evolving nuclear sequences [1] may be better suited to this particular biogeographic question.

Supplemental Information

Supplemental Information including supplemental results, methods and one figure can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.06.001.

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Reply to Phillips *et al.*

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Phillips *et al.* [1] reply to our finding that genetic divergence between subterranean metacrangonyctid amphipods from opposite shores of the Atlantic is congruent with vicariance by plate tectonics [2]. They highlight three presumed shortcomings in our analyses: first, the third codon positions of the mitochondrial genes used to reconstruct the metacrangonyctid phylogeny are saturated and consequently should be excluded from the analysis; second, substitution rates across the tree do not fit an uncorrelated lognormally distributed (UCLD) clock, and implementation of a random local clock (RLC) model would be more appropriate; third, the two dates that we used to calibrate the tree are fairly recent compared to the overall tree length, while the inclusion of a deep fossil calibrator could have improved dating. However, much of the criticism of Phillips *et al.* applies more to their modification of our data set than to the original data themselves. Specifically, their addition of several highly divergent taxa — driven by the necessity to include taxa encompassing the new deep calibration node they propose — largely alters the properties of our original data matrix. We maintain that third codon position saturation and deviation from lognormal rates largely apply to the new and not to the original data set. We also have some concerns about the fossil calibration used by Phillips *et al.* [1].

Both the stemminess metric calculated by Phillips *et al.* [1] and our Xia and Lemey test indicate that third codon positions are indeed more saturated than first and second positions. However, values for each of the three codon positions in our original dataset are lower than critical values [2], suggesting that there is still phylogenetic signal at third positions despite partial saturation. That third mitochondrial codon positions are partially saturated is no surprise and has been extensively demonstrated at various taxonomic levels [3]. However, the occurrence of partial saturation does not necessarily imply lack of phylogenetic signal and implementation of partitioning over codon positions and relaxed-clock models has been shown to improve molecular phylogenetic and dating analyses in such circumstances [4]. Notwithstanding, in order to evaluate the relevance of this argument analytically, we reanalyzed our data after exclusion of third codon positions and we show that this modification has a limited impact on age estimates (mean age 9% younger for node of the Atlantic clade) (Figure 1; Supplemental information).

Phillips *et al*. [1] also point out that rates are not lognormally distributed across our tree, although this seems to be mainly caused by the addition of distant outgroups [1]. In order to explicitly compare the two clock models (UCLD and RLC) in a formal phylogenetic Bayesian framework, we used the posterior simulation-based analog of Akaike's information criterion recently developed by Baele *et al*. [5]. The test indicates that UCLD clock, implemented in the original analysis, outperforms RLC (Supplemental information). For the sake of comparison, we nevertheless reanalyzed the original dataset, with and without third codon positions, applying a RLC model as suggested by [1]. New age estimates, although generally younger, still fall within the confidence age interval estimated using UCLD clocks (Figure 1), indicating that the original results are robust with respect to the use of different clock models and the effect of third codon positions.

Phillips *et al.* also refer to clock calibration issues [1]. We fully agree that, ideally, molecular clock calibrations are best implemented by deploying several well-dated fossils robustly assigned to particular nodes positioned at different timescales in a given phylogeny [3]. However, fossil calibrations in molecular phylogenies are far from being a silver bullet, for several reasons: fossils may be incorrectly assigned to the crown and not to the stem of a clade; fossils may be considerably younger than the origin of their respective clade; and data limitations may compromise both fossil taxonomic placement and dating [3,6]. Furthermore, the fossil record is notoriously incomplete, and in many instances appropriate

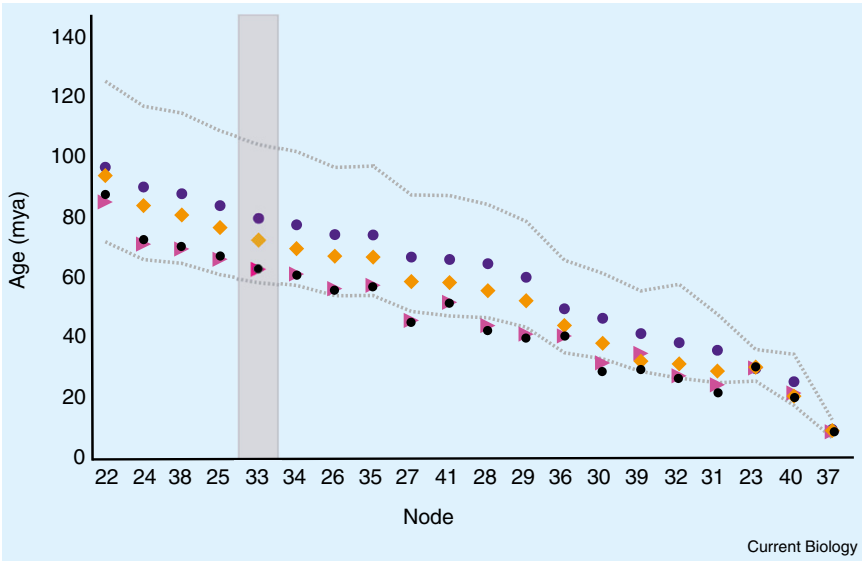

Figure 1. Estimation of node ages under relaxed lognormal and random local clocks.
Comparison of node age estimates obtained using different analyses. Common to all are the palaeogeographical events used for calibration, the phylogenetic tree and corresponding node numbers reported in [2] and the use of independent substitution models and clocks for each codon site. Original analysis (uncorrelated lognormal relaxed clock, third codon positions included): blue dots represent mean estimates, gray lines represent 95% HPD intervals. Data reanalysis: orange squares represent mean estimates applying an uncorrelated lognormal clock model with third codon positions excluded; pink triangles with random local clocks and third codon positions included, black dots with random local clocks and third codon positions excluded. The key node linking both sides of the Atlantic in the phylogeny reported in [2] is highlighted with a grey box.

fossil calibrators are simply not available. In fact, the fossil record of the Amphipoda is extremely poor, with the oldest fossils known corresponding to casts preserved in Eocene Baltic amber no older than 54–40 million years (my) [7] (Supplemental information). Without an appropriate fossil calibrator for the taxa under investigation, Phillips *et al.* [1] add a number of non-metacrangonyctid amphipod, isopod, decapod and hoplocarid outgroups to our original alignment [1], in order to encompass an inferred date for the separation of the Subclasses Eumalacostraca and Hoplocarida derived from fossil information. In doing so, they introduce two possible problems. This is an extremely deep node in relation to our ingroup, and raises significant concern over the overall rate stability. Furthermore, they used the age of *Hesslerella* to date the split of the Subclasses Eumalacostraca and Hoplocarida, seemingly overlooking that *Hesslerella* is an undeniable crown-group phreatoicidean [8,9], a member of the Peracarid order Isopoda, and as such its age (325 mya) should be considered a *minimum* constraint age for the Peracarida. Thus, it should be assigned to the node Isopoda/Amphipoda in Phillips *et al.*'s [1] tree instead of to the Hoplocarida/Eumalacostraca node. It is important to mention here that the Eumalacostraca includes three Superorders: Syncarida, Eucarida (to which the decapods belong) and Peracarida, the latter including the amphipods and Isopods, among other groups (Supplemental information).

The calibration of molecular clocks and deduction of subsequent evolutionary timescales have always been open to debate and discussion [3,6]. We acknowledge that there are a number of assumptions and parameters that can be applied to both phylogeny estimation and molecular clock calibration that can have an impact on the resulting estimates. A full understanding of the relative importance of vicariance and dispersal to explain the distribution of metacrangonyctid amphipods would require taxonomically well-sampled, robust multi-loci phylogenies of the lineages forming the superorder Peracarida, with reliable and appropriately distributed palaeographic and fossil calibrations.

The criticisms of Philips *et al.* [1] demonstrate the power of parameter choice to drive biogeographic inference. Their differing results are largely driven by the modification of the original dataset and a possibly inappropriate deployment of fossil calibration. Potentially powerful parameters do indeed carry great responsibility.

Supplemental Information

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